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Effect of different Potting Mixtures on Acclimatization of *In vitro* Developed Plants on Survival Percentage under Glass House Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The experiment was conducted during the years 2020-22, all of the experiments for the current research were carried out in the Plant Tissue Culture Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Gwalior, Rajmata Vijayaraje Scindia Krishi Vishwa

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Vidyalaya, Gwalior (M. P.). Pomegranate cv. Bhagwa plantlets were placed in polythene bags containing various potting mixtures, such as soil, FYM, and Vermicompost, alone and in various combinations. The hardened plantlets were then exposed to direct sunshine to help them acclimate to their new surroundings. The percentage of tissue culture plantlets that survived was calculated. Experiments were conducted in a Completely Randomized Design (CRD) with four replications of each treatment and each replication contain three explants. Data were analyzed using Duncan's multiple range test whereas the survival percentages were analysed by arc-sine transformation. On the basis of present study it is concluded that cocopeat soil combination had the highest survival rate (95.00%), followed by cocopeat (85%). In the case of pomegranate, sand, perlite, vermiculite, vermiculite soil and perlite sand alone were not shown to be effective hardening materials because all of the plants withered within a week. After two months of hardening under polyhouse circumstances, cocopeat soil showed the greatest growth with a notable increase in plant height (37.23 cm), followed by cocopeat (33.03 cm). Therefore, we may conclude that cocopeat is the ideal material for pomegranate plants that have been produced *in vitro*.

Keywords: Survival; pomegranate; cocopeat; hardening; molecular and polyhouse.

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is an economically important species of the tropical and subtropical regions of the world owing to its delicious edible fruits and pharmaceutical and ornamental usage [1]. It is native to Persia and has been widely cultivated throughout drier parts of Southeast Asia, Malaysia, the East Indies tropical Africa and India [2]. In India, it is found from Kanyakumari to Kashmir but commercially cultivated only in Maharashtra *i.e.,* accounts 71.2 per cent of the total area in India and 66.93 per cent of the total production in the country.

The pomegranate is a small tree, having height of 20 or 30 ft with glossy and leathery leaves and it bears flowers with red, fleshy, tubular calyx which persists on the fruit. Fruit is generally used in flavouring of mixed drinks, ice creams or desserts. However, pomegranate also possesses a number of medicinal properties. The juice is useful in the cure of leprosy rind of the fruit is useful in curing dysentery and diarrhea. The coloring matter present in the fruit rind is also used in the synthesis of dyeing material for clothes. Pomegranate is a rich source of carbohydrate (14.5%), protein (1.6%), fat 10 mg/100 g, calcium (10 mg/100 g), phosphorus (70 mg/100 g), iron (0.3 mg/100 g) and vitamin C (65 mg/100 g). It is also rich in riboflavin, niacin, ascorbic acid and potassium.

India is the world's leading country in pomegranate production. The estimated area under pomegranate was 262 thousand ha [3] with total production 3034 thousand MT [3]. Micropropagation in pomegranate would help in overcoming difficulties of vegetative propagation, producing true to- type plants along with rapid mass production and disease-free planting materials [4,5]. Also, the demand for the good quality planting material has been steadily increasing in recent years. The enormous potential in the domestic market can be revitalized by widening the spectrum of micropropagation in pomegranate crop.

However, information regarding establish a protocol for *in vitro* mass multiplication of pomegranate plants in Madhya Pradesh is lacking. Keeping in view the above discussed facts of sufficient information and sparce related research, the present investigation was undertaken under Gwalior conditions.

2. MATERIALS AND METHODS

The experiment was conducted during the years 2020-22, all of the experiments for the current research were carried out in the Plant Tissue Culture Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Gwalior, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M. P.).

2.1 Source of Explants

Pomegranate cv. Bhagwa explants were obtained from the Horticulture Nursery of the College of Agriculture in Gwalior (M.P.)

2.2 Chemicals

Hi-Media provided all of the major, minor and other substances required for the creation of media, such as vitamins and plant growth regulators.

2.3 Glassware and Equipment

The studies were carried out using BOROSIL brand borosilicate glassware. Wide mouth jam bottles (6 cm diameter x 13 cm height) with autoclavable polypropylene closures were utilized for nutritional research of various cultures. Test tubes measuring 25 mm x 150 mm were also used. Erlenmeyer flasks and beakers, as well as a Hi- Media micro-pipette, were utilized for stock solution preparation, media preparation, and other tasks. For aseptic manipulations, consumables such as forceps, scalpels, needles, and spatulas were employed.

2.4 Washing of Glass Wares

Glass wares were washed in a Tween-20 (0.1 %) solution overnight. After that, the cleaning solution was entirely eliminated by vigorously washing with tap water. Finally, the glassware was washed in distilled water and dried in a 60-80°C oven; clean glassware was protected from dust.

2.5 Sterilization of Glass Wares

Non-absorbent cotton or caps were used to plug test tubes and flasks, while polypropylene caps were used to shut culture bottles. Aluminum foil was used to wrap pipettes and Petri dishes. All of the glassware was autoclaved for 15 minutes at 15 psi and 121°C. They were then placed in a hot air oven for two hours at 80-100°C. Forceps, scalpels and needles used in aseptic operations were sanitized by soaking them in 100% ethanol, then burning and chilling them.

2.6 Hardening of Tissue Culture Plantlets

To remove agar medium stuck to roots, *in vitro* produced plantlets were removed from culture bottles and cleaned with distilled water. For primary hardening, the plantlets were dipped in 0.5 percent Bavistin for 5 minutes before being planted in plastic pro-trays containing cocopeat. For 30 days, these plateswere cultivated in a polyhouse with 70% humidity and a temperature range of 25-27°C. After that, the plantlets were moved to a shade net house for secondary hardening. Plantlets were placed in polythene bags containing various potting mixtures, such as soil, FYM, and Vermicompost, alone and in various combinations and are given in Table 1. During the next 45 days, they were kept at 50% light intensity by careful administration of water and soluble nutrients. The hardened plantlets were then exposed to direct sunshine to help them acclimate to their new surroundings. The percentage of tissue culture plantlets that survived was calculated.

2.7 Experimental Plan and Design

Experiments were conducted in a Completely Randomized Design (CRD) with four replications of each treatment and each replication contain three explants. Data were analyzed using Duncan's multiple range test whereas the survival percentages were analysed by arc-sine transformation.

Table 1. Combination of different potting mixtures

Treatments	Mixture Composition
T_1: Sand	-
T_2: Soil	-
T_3: FYM (Farm Yard Manure)	-
T_4: Perlite	-
T_5: Vermiculite	-
T_6: Vermicompost	-
T_7: Cocopeat	-
T_8: Cocopeat: Soil	1:1
T_9: Vermiculite: Soil	1:1
T_{10}: Perlite: Soil	1:1
T_{11}: Perlite: FYM	1:1
T_{12}: Sand: Soil	1:1
T_{13}: Cocopeat: Vermiculite	1:1
T_{14}: Sand: Vermicompost	1:1
T_{15}: Sand: Soil: FYM	1:1:1

3. RESULTS AND DISCUSSIONS

3.1 Hardening

The most significant and fundamental stage in a micropropagation programme, successful acclimatization of tissue grown plants under field conditions dictates the direction of future planning. Here, the best acclimation of plantlets was studied across fifteen different potting mixtures. We used *in vitro* produced plants that have been two months old (5.0-6.0 cm in length with 7-8 roots). Table 2 and Fig. 1 revealed that the cocopeat soil combination had the highest survival rate (95.00%), followed by cocopeat (85%). In the case of pomegranate, sand, perlite, vermiculite, vermiculite soil and perlite sand alone were not shown to be effective hardening materials because all of the plants withered within a week. After two months of hardening

under polyhouse circumstances, cocopeat soil showed the greatest growth with a notable increase in plant height (37.23 cm), followed by cocopeat (33.03 cm). Therefore, we may conclude that cocopeat is the ideal material for pomegranate plants that have been produced *in vitro*. Similar findings were also reported by Deepika and Kanwar [6]; El-Agamy et al. [7]; Helaly et al. [8]; Kaji et al. [9]; Patil et al. [10]; Dev et al. [11]; Rahman et al. [12].

Table 2. Effect of different potting mixtures on acclimatization of *in vitro* developed plants in glass house

Treatments	Survival Percentage			Plant height (cm)		
	20 days	40 days	60 days	20 days	40 days	60 days
T_1: Sand	0 (0)	0 (0)	0 (0)	0.00^{c}	0.00^{c}	0.00^{c}
T_2: Soil	35 (36.27)	35 (36.2)	0 (0)	2.80^{bc}	6.83^{bc}	0.00^{c}
T_3: FYM (Farm Yard Manure)	45 (42.13)	45 (42.1)	0 (0)	2.93^{bc}	7.16^{bc}	0.00^{c}
T_4: Perlite	0 (0)	0 (0)	0 (0)	0.00^{c}	0.00^{c}	0.00^{c}
T_5: Vermiculite	0 (0)	0 (0)	0 (0)	0.00^{c}	0.00^{c}	0.00^{c}
T_6: Vermicompost	65 (53.72)	65 (53.7)	65 (53.7)	5.46^{bc}	14.03^{abc}	21.30^{b}
T_7: Cocopeat	85 (67.21)	85 (67.2)	85 (67.2)	7.83^{bc}	18.76^{ab}	33.03^{ab}
T_8: Cocopeat: Soil	95 (77.07)	95 (77.0)	95 (77.0)	12.26^{a}	24.16^{a}	37.23^{a}
T_9: Vermiculite: Soil	0 (0)	0 (0)	0 (0)	0.00^{c}	0.00^{c}	0.00^{c}
T_{10}: Perlite: Soil	0 (0)	0 (0)	0 (0)	0.00^{c}	0.00^{c}	0.00^{c}
T_{11}: Perlite: FYM	45 (42.13)	0 (0)	0 (0)	2.96^{bc}	0.00^{c}	0.00^{c}
T_{12}: Sand: Soil	55 (47.86)	55 (47.8)	55 (47.8)	0.00^{c}	12.73^{abc}	20.33^{b}
T_{13}: Cocopeat: Vermiculite	75 (60.00)	75 (60.0)	0 (0)	5.50^{bc}	13.33^{abc}	0.00^{c}
T_{14}: Sand:Vermicompost	35 (36.27)	35 (36.2)	0 (0)	9.83^{bc}	7.46^{bc}	0.00^{c}
T_{15}: Sand: Soil: FYM	75 (60.00)	75 (60.0)	75 (60.0)	0.00^{c}	13.06^{abc}	21.43^{b}

Fig. 1. (a) *In vitro* plants ready for hardening (b) and (c) Hardening in different potting mixtures

4. CONCLUSIONS

From the above overall study, it is concluded that cocopeat soil combination had the highest survival rate (95.00%), followed by cocopeat (85%). In the case of pomegranate, sand, perlite, vermiculite, vermiculite soil and perlite sand alone were not shown to be effective hardening materials because all of the plants withered within a week. After two months of hardening under polyhouse circumstances, cocopeat soil showed the greatest growth with a notable increase in plant height (37.23 cm), followed by cocopeat (33.03 cm). Therefore, we may conclude that cocopeat is the ideal material for pomegranate plants that have been produced *in vitro*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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